RADIALLY ORIENTED CLEAVAGE IN BLASTULAE AND IN GASTRULAE OF THE STARFISH, ASTERIAS FORBESI

GEORGE E. WHEELER

Biology Department, Brooklyn College, Brooklyn, N. Y.

Orientation of the mitotic spindle and of the cytoplasmic cleavage plane are generally of greater interest to the plant histologist than to those working with animal tissues. Although the earlier zygotic divisions have been studied at length, only an occasional investigator has considered patterns of division in connection with later developmental events (*e.g.*, Pohley, 1959; Lipp, 1959). Highly oriented divisions seem particularly likely to occur during the formation of starfish blastulae and gastrulae: the arrangement of cells in just one layer could be explained, at the first level, by radial cleavage planes. This aspect of development seems to have received less attention than either the earlier or the later phenomena.

Embryologists of the late nineteenth and of the early twentieth centuries discussed in great detail the arrangements of cleavages up to the 16- and to the 32-cell stages. They frequently used such terms as "meridional" and "equatorial" to describe the division planes (*e.g.*, Seeliger, 1892; Boveri, 1901). Zeigler (1924) gave a particularly precise account, for several echinoderms, of the "vertical" and of the "horizontal" cleavages which bring the zygote to 8, to 16, and to 32 cells. Korschelt (1936) attributed the several basic patterns (radial, spiral, and bilateral) to cleavage plane direction. He too used "meridional" and "equatorial" for divisions up to the 32-cell stage. Obviously, the terms "meridional," "equatorial," "vertical," and "horizontal" imply divisions which cut the zygote in the same way that radial cleavages cut the blastula.

Metschnikoff (1885: see his Figs. 57–63) pictured blastulae with radially dividing blastomeres, and described the cleavages as being radial (his p. 664). He said nothing about maintaining a single layer of cells, but his figures show this characteristic. In a discussion of the coeloblastula, Korschelt (1936) mentioned radial cleavage (p. 93), one-layered wall (p. 94), and the production of the several-layered condition by tangential cleavages (p. 95); however, he did little more than remark on these conditions in passing.

Except for a photomicrograph in Immer's (1957) paper on cytochemical aspects, the only recent reference I have found is that of Wolpert and Gustafson (1961) on sea urchin blastulation. Observations on living embryos led these authors to stress the importance of radial cleavages in maintaining the single layer of cells. They considered this restricted orientation (p. 381) ". . . an essential feature in blastula formation."

My observations were made on fixed, sectioned, and stained blastulae and gastrulae. Numerous mitotic figures indicated that radial cleavage is the rule, not only through blastulation but during gastrulation as well. Additional phenomena of interest, found in this same material, include occasional cleavages

passing along the longer axis of the cell, and additional patterns of division figure orientation.

Materials and Methods

Dr. Evelyn Rosenberg, New York University Medical School, supplied the several collections of *Asterias forbesi* used in this study. I am very happy to acknowledge Dr. Rosenberg's generosity and consideration over the past several summers.

The embryos were fixed in 10% formalin. They were pipetted into the emptied pupal sacs of ants, and the open end of each sac was tied shut with a hair. This kept the embryos together during the subsequent processing, which included dehydration by the tertiary butyl alcohol method (Johansen, 1940) and embedding in Tissuemat, melting point ca. 55° C. Sections were cut at 5, at 7, or at 9 micra. Different thicknesses had specific advantages and disadvantages: thinner sections gave clearer pictures, both visual and on film; thicker sections simplified the interpretations of doubtful orientations and included a greater number of whole mitotic figures per single section.

A number of staining techniques produced excellent preparations: Heidenhain's iron hematoxylin, Capinpin's brazilin (both given in Johansen, 1940), Einarson's gallocyanin (given by Terner and Clark, 1960), and azure A-Schiff. The latter was used as suggested by Himes and Moriber (1956) with 10 minutes' hydrolysis in 1 N HCl at 60° C. This stained practically nothing but the chromosomes. The other techniques showed the spindles clearly and also stained the cytoplasm.

Photomicrographs were taken with a Leitz Wetzlar "Ortholux" microscope equipped with a Leitz Wetzlar "Orthomat" camera attachment.

Observations

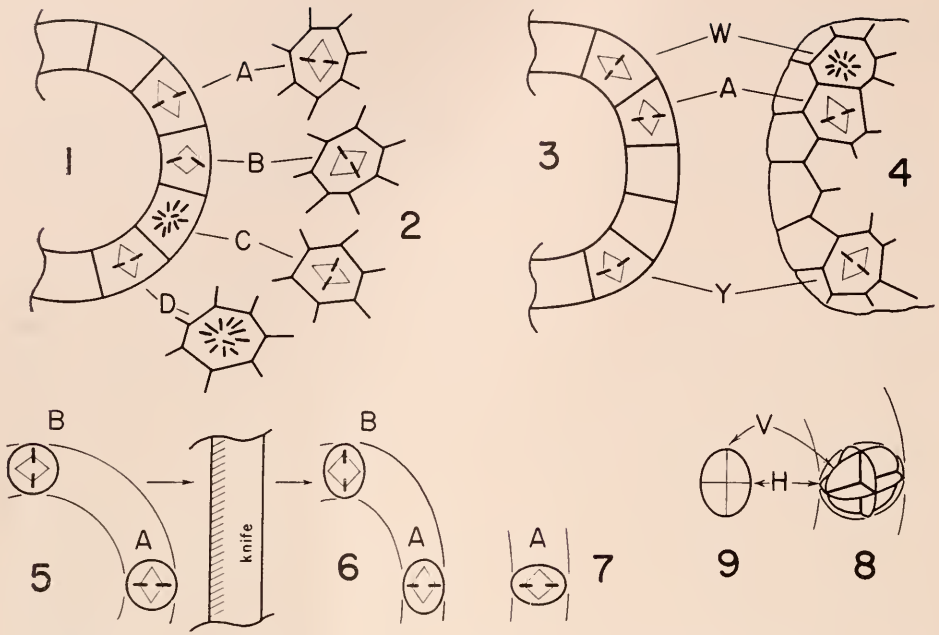
Interpretation of mitotic figure orientation

A thin section may include any level of the sub-spherical blastula, so it is difficult at times to interpret the mitotic spindle position in relation to the blastula as a whole. This is of paramount importance, however, since potential radial cleavages may be inferred only from certain alignments of the spindle and of the chromosome clusters.

A median section of the blastula appears as a ring of cells. Within these cells, the spindles may lie in various positions which are all consistent with division along the radial plane. In Figure 1, cell *A* shows the spindle in equatorial view; in cell *B*, it is in oblique view; in cell *C*, it is in polar view. All three have a tangentially oriented spindle axis leading to radial cleavage. In contrast, a radially oriented spindle axis with tangentially cleaving cytoplasm and the consequent production of two layers of cells would require the alignment shown in cell *D*. Figures 10–18 are photomicrographs of the first three arrangements.

Figure 2 shows cells *A*, *B*, *C*, and *D* of Figure 1 as they would appear in face view on one side (tangential section) of a blastula (see Figs. 19–24). The difference between an orientation leading to radial cleavage (cells *A*, *B*, *C*) and that leading to a tangential cleavage (cell *D*) is clear in these cases, but the two types are not always so easily distinguished.

Since serial sections contain slices ranging from a true median section, through



FIGURES 1–4. Interpretation of spindle orientations relative to radial and to tangential cleavages. Diagrammatic. Figure 1 represents the mid-section of a blastula. The spindle positions of cells *A, B,* and *C* lead to radial division; they appear, respectively, in equatorial view, in oblique view (between equatorial and polar), and in polar view. In cell *D,* the spindle lies in radial orientation which would lead to tangential cleavage. Figure 2 shows the same spindles as they would appear in face view on a tangential section (one side) of the blastula. Figures 3 and 4 show the possible misinterpretation of spindle orientation if one side of the blastula is somewhat flattened due to pressure of an adjacent embryo. The spindle in cell *W* is really aligned for radial cleavage (Fig. 3) but would be misinterpreted as producing a tangential division (Fig. 4) because of the acute curve of the blastula at the edge of the section. Similarly, the spindle of cell *Y* would be misinterpreted as producing a radial cleavage (Fig. 4) whereas it is really aligned to accomplish tangential cleavage (Fig. 3).

FIGURE 5. Two cells, *A* and *B,* assumed to be spherical, before sectioning. The arrows indicate the direction the paraffin block moves as it passes the knife.

FIGURE 6. The same two cells showing compression due to sectioning. Cell *B* would appear to be cleaving along its longer axis, but this is an artifact resulting from compression (compare with Fig. 5). Cell *A* would be similarly distorted but would seem to be cleaving along its shorter axis.

FIGURE 7. If, despite sectioning, a cell in the same position as *A* still has a longer radial diameter and is cleaving radially, it may be interpreted as cleaving lengthwise (see text).

FIGURE 8. A blastomere cut along the three planes of sectioning, which pass through the three cell axes. The three axes are assumed to be unequal, so that each plane has a longer and a shorter diameter (see text).

FIGURE 9. The blastomere of Figure 8, shown in face view as it would appear on a tangential section (*e.g.,* Fig. 4) of a blastula. The difference in length between the two visible axes is clear; the third axis lies perpendicular to the page (see text).

cells closer and closer to the sides, and finally the actual side (tangential section) of the embryo, and since the blastula may be deformed by pressure of adjacent embryos, some orientations require careful interpretation. The spindle may seem to be aligned radially but actually lie along the expected tangent. Figure 3 shows

a blastula somewhat flattened on one side (due, presumably, to pressure of an adjacent embryo). At cell *W*, the wall is curved abruptly so that this particular cell is likely to be included in a side (tangential) section. Although its spindle is oriented for a radial cleavage, as is clear from Figure 3, it would present an almost polar view on a tangential section (Fig. 4) and in consequence be identified incorrectly as a tangential cleavage. Figures 25 and 26 show this condition in actual cells.

In a similar fashion, it is possible to misinterpret a radially aligned spindle (tangential cleavage) as lying in the tangential direction: *e.g.*, the spindle in cell *Y*, Figure 3, would seem to be so oriented on a tangential section (Fig. 4).

The cases just described are more easily interpreted on thicker sections. Those found on thinner sections, however, can usually be worked out by study of the preceding and of the succeeding serial sections.

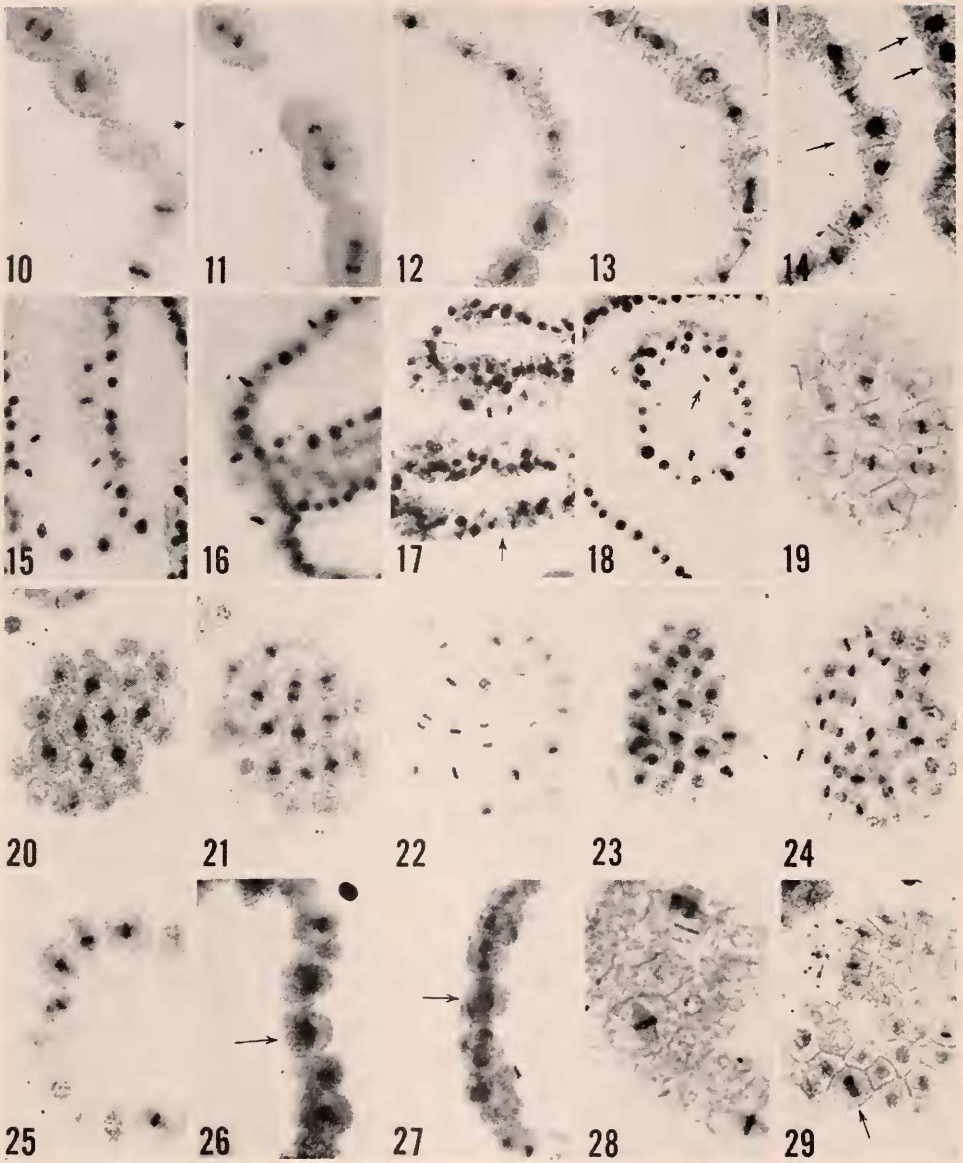
Radial cleavage (tangential spindle orientation)

With few exceptions, the hundreds of mitotic figures observed were definitely identified as leading to radial cleavage of the blastomeres, relative to the blastular sphere. Interpretation of some spindles was uncertain. Such cases might constitute real exceptions, perhaps associated with other developmental events such as mesoderm formation. On the other hand, they could be divisions in the expected radial plane, but occurring in cells whose positions were peculiarly altered during processing or sectioning.

Although mitosis is no longer synchronous in the blastula stages studied, it is not unusual to find clusters of mitotic cells on the same embryo. Single medial sections often include arcs of two or more dividing cells, all lying in equatorial or in other aspects (Figs. 10–14). Tangential sections (one side of the embryo) may include from several to as many as ten or even more mitotic figures, oriented for radial cleavage (Figs. 19–24). On some tangential sections, mitotic figure positions, *relative to each other*, suggest yet another level of patterning in addition to that already described (Figs. 20, 21, 23). Arrays of this type will be discussed later.

The starfish gastrula also consists of layers one cell thick; therefore, a spindle orientation comparable to that of the blastula may be expected. Since the concept of radial cleavage loses meaning as invagination and gastrocoel formation proceed, comparable evidence for this type of division is found in cleavages perpendicular to the sheet of cells, *i.e.*, perpendicular to a tangent to the curved sheet at that point. Such divisions will maintain a single-layered ectoderm and a single-layered endoderm. Problems relating to spindle figure orientation found throughout sections of gastrulae are much like those encountered in the blastula studies. In these later embryos, however, such factors as the more complex form of the gastrula and the reduced cell size increase the difficulty of interpretation.

Observations on later gastrulae are further complicated by the temporarily changed location of the cleaving cell. It rounds up toward the surface of the cell layer, shifting toward the outer surface of the embryo in the case of the ectoderm, and toward the gastrocoel in the case of the endoderm (Figs. 15–18). The spindle and chromosomes are similarly displaced, so that on tangential sections (sections including one side of the gastrula) the mitotic figure is not in focus



FIGURES 10–14. Radial sections of early, of mid-, and of late blastulae, showing spindles aligned for radial cleavage (compare Fig. 1, cells *A, B, C*). Most spindles are in equatorial view, but there are several in oblique aspect. In Figure 14, the arrows point to spindles seen in polar view.

FIGURES 15–17. Median longitudinal sections of gastrulae, showing radial cleavages. In Figures 15 and 17, anaphasic or telophasic spindles project toward the archenteron, above the level of the nuclei in other cells of the endoderm. In Figures 16 (above) and 17 (see arrow), mitotic figures of the ectoderm project out beyond the other nuclei. This phenomenon is obscured in Figure 17 due to a mild distortion, presumably resulting from pressure of another

at the same level as the interphase nuclei of the adjoining cells. Metschnikoff (1885) indicated this displacement in several of his drawings, and Wolpert and Gustafson (1961) published a photomicrograph of the phenomenon occurring in a living cell (their Fig. 4).

Despite the difficulties just enumerated, the positions of most mitotic figures could be determined satisfactorily: the spindle lies parallel to the plane of the cell layer, *i.e.*, it determines a cleavage perpendicular to the cell sheet. Even cells lying in the curved surface at the blastopore cleave at right angles to the tangent at that point (Fig. 16). The same orientation prevails at the opposite end of the archenteron.

The possibility of cleavage along the long axis of the blastomere

Later blastulae occasionally include a mitotic cell in which the tangential axis is shorter than the radial. Since the spindle axis lies tangentially, as expected, the cleavage furrow in such a cell would have been radial, and therefore would have followed the longer cell diameter. If, despite fixation, dehydration, etc., this is a true picture of the cell, then the cleavage in such cases is a violation of Hertwig's rule (Wilson, 1925, p. 984).

Paraffin sections are somewhat compressed during sectioning; as a consequence, only cells in certain positions may be considered as possibly undergoing longitudinal cleavage. Figure 5 shows two spherical blastomeres before sectioning. Figure 6 shows the distortion to be expected if the blastula passes the knife in the direction indicated. All blastomeres are compressed so that their long

embryo. In Figure 16 (below), a metaphasic cell lies in the curve of the blastopore rim and shows proper orientation for a radial cleavage.

FIGURE 18. Transverse section of a gastrula showing a metaphasic spindle lying toward the archenteron (below). The arrow points to what is really a slightly oblique polar view of a potential radial cleavage; one of the chromosome clusters (above) is somewhat out of focus. Two additional mitotic cells are out of focus but still visible, one at the left and one at the right in the endoderm.

FIGURES 19–24. Tangential sections of early and later blastulae and gastrulae, showing spindles oriented for radial cleavage (compare Fig. 2, cells *A, B, C*). An additional level of orientation is evident in Figures 20, 21, and 23, wherein most of the spindles are aligned relatively parallel to each other (see text).

FIGURE 25. Possible misinterpretation of spindle orientation. All mitotic figures, except the one directly above, are *seemingly* aligned for tangential cleavages. This interpretation, however, is incorrect, and is due to their being in a section which lay adjacent to the side of the blastula, as is indicated by the portion of a cell lying just inside the circle of cells. These cleavages are really radial (compare Figs. 3, 4, and see text).

FIGURES 26, 27. The arrows point to radial cleavages which would cut through a longer cell diameter (see Figs. 5–7). In Figure 26, the arrow also points to the bounding membrane (faint line) on the left of the blastomere. The cell above, in Figure 26, shows a pseudo-tangential division similar to those in Figure 25.

FIGURES 28, 29. Longitudinally dividing blastomeres seen in tangential sections of the blastulae. Despite its being in anaphase, the spindle of the cell in Figure 29 would lead to a cleavage along the longer diameter. The sections of Figures 26–29 were all cut as the block passed the knife from side to side (with reference to the page; see Figs. 5–7).

Staining: Figures 10, 11, 17, 23, 24, 27 were stained with gallocyanin; 12, 13, 14, 19, 20, 21, 25, 26, 28, 29 with hematoxylin; 15, 16, 18, 22 with azure A-Schiff.

Magnification: Figures 13, 14, × 480; Figure 19, × 300; Figure 27, × 550; Figure 28, × 775; all remaining, × 400.

axes are parallel to the knife edge. Cell *B* appears to be cleaving lengthwise; this, however, is obviously a pressure artifact, because the cell was spherical before sectioning (compare Figs. 5 and 6). On the other hand, cell *A* would cleave along what appears to be its shorter, *i.e.*, its radial axis. But if, despite sectioning pressure, a mitotic cell in position *A* still has a longer radial diameter, and if its spindle is tangentially oriented, then it is reasonable to assume that the cell was cleaving along a longer axis at the time of fixation (Fig. 7). Cells located between *A* and *B* could also be interpreted as dividing lengthwise as long as their spindles were oriented roughly parallel to the knife edge. Figures 26–29 are examples of such cells.

The three diameters of the blastomere, two tangential and one radial (Fig. 8), may all be unequal in length, with the radial being longest. If this condition obtains, then lengthwise cleavage may appear in either of two forms. These are shown in Figure 8 which represents such a blastomere. The division plane may follow the shortest, and one of the longer cell axes (horizontal cleavage plane, *H*, Figs. 8, 9); or the division plane may follow both longer axes (vertical cleavage plane, *V*). Since only two cell axes lie in the plane of a paraffin section, with the third projecting into the depth dimension, it is virtually impossible to determine which of these two types of planes is exemplified by a particular cleavage furrow. It is evident from Figure 9, however, that the tangential diameters may be compared on a tangential section of the blastula. Figures 28 and 29 are face views of lengthwise cleavages. Here again it is impossible to determine whether the depth diameter (radial diameter) is longer or is shorter than the two tangential diameters.

Inferences based on estimated or measured dimensions of blastomeres in paraffin sections are open to serious question. This is especially critical since cells undergo a decided change in shape during division (Wolpert and Gustafson, 1961; see their Figure 4, and their p. 381). Addition of a fixing fluid, subsequent processing, or both, might prevent a typical shape change or might produce distortions of the normally cleaving cell. Whatever the dangers of interpretation from fixed material may be, it is interesting that a suggestion of lengthwise cleavage may be observed in blastomeres as late as anaphase (Fig. 29).

General Discussion

Wolpert and Gustafson (1961) stressed the role of radial cleavage in generating and in maintaining a single layer of cells. This "first order" of patterning, however, merely restricts the number of layers to one. Other organisms, also built of single layers, may take the form of tubes or of bladders, sometimes with opposite sides appressed together (see Bonner, 1952; his Figure 79 and p. 24). It seems reasonable to infer, then, the existence of an additional level of mitotic spindle orientation which is responsible for the sub-spherical shape of the blastula.

The initial divisions of the zygote, alternating between "meridional" and "equatorial," show a pronounced regularity only to the 8-, to the 16-, or to the 32-cell stage (Seeliger, 1892; Boveri, 1901; Korschelt, 1936). Nevertheless, a single-layered sphere could be developed from this early pattern, if subsequent divisions were to "average out" in various directions around the surface.

It is not clear how this averaging might be accomplished. Completely random

divisions (cleavages distributed equally among all possible radial planes cutting through the sphere) could maintain a spherical blastula. Such a pattern, however, may not actually occur. Many sections show a surprising number of spindle axes aligned in essentially the same direction (Figs. 20, 21, 23); frequently, there is a suggestion of two, mutually perpendicular systems of cleavages (Fig. 19), yet other arrangements vaguely suggest some further type or types of ordering. These may all be manifestations of another mechanism, namely, a complex, overall plan of subdivision which, while somewhat variable about the surface of the blastula, is definite and consistent at the level of the whole embryo.

Although either of the two systems of cleavage just described could generate a spherical blastula after the initial divisions, the second type is likely to prevail, at least in a general form, after invagination begins. The gastrula shows a decided elongation, which may depend on a significantly higher number of cleavages transverse to the length axis of the embryo. These would yield pairs of daughter cells aligned lengthwise, thereby increasing the length dimension. Many mitotic figures, not only in the ectoderm but in the gastrocoel wall as well, display the required orientation for transverse cleavage (Figs. 15, 16, 17).

The concept of an additional degree of mitotic spindle orientation in the blastula and in the gastrula could be confirmed by observation. If it is established, then the following questions become meaningful: what mechanism or mechanisms control spindle orientation, and how does the mechanism or the mechanisms operate? Beyond noting that the organism as a whole exerts control over its developing parts, there seems to be little that can be said in answer to such questions at the present time.

Summary

Blastulae and gastrulae of the starfish, *Asterias forbesi*, were fixed, run into paraffin, sectioned, and stained to show the mitotic figures. These were found to lie in a tangential direction, thus leading to radial cleavages in the blastulae, and to their equivalent (*i.e.*, cleavage perpendicular to a tangent to the curved surface) in the gastrulae. The single layer of cells characterizing these stages is therefore the result of spindle figure and of cleavage plane orientations. Other observations suggested that the spindles may lie in definite arrangements relative to each other. Several patterns were found, and their relationship to blastula and to gastrula formation is briefly discussed. There were indications that the blastomeres may, though rarely, undergo cleavage along a longer diameter.

LITERATURE CITED

Bonner, J. T., 1952. Morphogenesis. Princeton University Press, Princeton, N. J.

Boveri, T., 1901. Die Polarität von Ovocyte, Ei und Larve des *Strongylocentrotus lividus*. *Zool. Jahrb. Anat.*, **14**: 630–653.

Himes, Marion, and L. Moriber, 1956. A triple stain for desoxyribonucleic acid, polysaccharides and proteins. *Stain Technol.*, **31**: 67–70.

Immers, J., 1957. Cytochemical studies of fertilization and first mitosis of the sea urchin egg. *Exp. Cell Research*, **12**: 145–153.

Johansen, D. A., 1940. Plant Microtechnique. McGraw-Hill Book Co., Inc., N. Y.

Korschelt, E., 1936. Vergleichende Entwicklungsgeschichte der Tiere. Gustav Fischer, Jena.

Lipp, Christine, 1959. Cytologische Untersuchungen zum Kompensationsprinzip nach Henke am Flügel von *Ephestia kühniella* Z. *Biol. Zentralbl.*, **78**: 1–21.

Metschnikoff, E., 1885. Vergleichend-embryologische Studien. Ueber die Bildung der Wanderzellen bei Asteriden und Echiniden. *Zeitschr. wiss. Zool.*, **42**: 656–673.

Pohley, H. J., 1959. Über das Wachstum des Mehlmottenflügels unter normalen und experimentallen Bedingen. *Biol. Zentralbl.*, **78**: 232–250.

Seeliger, O., 1892. Studien zur Entwicklungsgeschichte der Crinoiden (*Antedon rosacea*). *Zool. Jahrb. Anat.*, **6**: 161–444.

Terner, J. Y., and G. Clark, 1960. Gallocyanin-chrome alum. I. Technique and specificity. *Stain Technol.*, **35**: 167–177.

Wilson, E. B., 1925. The Cell in Development and Heredity. Third ed. The Macmillan Co., N. Y.

Wolpert, L., and T. Gustafson, 1961. Studies on the cellular basis of morphogenesis of the sea urchin embryo. *Exp. Cell Research*, **25**: 374–382.

Ziegler, H. E., 1924. Beiträge zur Entwicklungsgeschichte der Echinodermen. *Zool. Jahrb. Anat.*, **46**: 521–572.